RFS# 011

STUDIES TO DETERMINE THE REPRODUCTIVE SUCCESS OF HATCHERY SPAWNERS (FCRPS BiOp Action #182)

SUBMITTED TO:

Bonneville Power Administration

SUBMITTED BY:

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PROJECT TITLE:

COMPARATIVE REPRODUCTIVE SUCCESS OF WILD AND HATCHERY ORIGIN SPRING/SUMMER CHINOOK SALMON THAT SPAWN NATURALLY IN THE PAHSIMEROI AND UPPER SALMON RIVERS

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PROJECT SUMMARY

The Idaho Department of Fish and game (IDFG) and the University if Idaho (UI) are collaborating in response to the Bonneville Power Administration's request for proposals addressing the NMFS Biological Opinion RPA 182. We propose a multiphase, comprehensive evaluation project to measure reproductive success and influence of hatchery origin salmonids upon wild fractions of the same population. Compelling evaluation of hatchery influence upon wild salmonids is a complex problem requiring substantial preparation since generation times are long and variable within a cohort, and individuals are both semelparous and r-selected. Additionally, long-term evaluation of hatchery influence upon a salmonid population through the F_2 generation requires much forethought for predicted returns to make statistically valid comparisons throughout the lifespan of the project (i.e. there will be enough projected returns in coming years to evaluate statistically). Accordingly, an investigation of this scope would be most cost effective provided the infrastructure for such an investigation were already in place. Likewise, it would be advantageous to integrate this largely genetic investigation with an ongoing, long-term project that can provide both logistical support for the collection of samples and collateral information regarding ecological and population dynamics within the same system.

In 2002, the IDFG and the UI, funded in part under the Idaho Supplementation Studies (ISS) project, began behavioral and genetic investigations on the Pahsimeroi River to evaluate the reproductive success of natural-origin and wild-spawning hatchery-origin chinook salmon through the F_1 generation. The hatchery origin recruits returning to Pahsimeroi River originated from supplementation broodstock, constructed from adult hatchery origin and naturally produced chinook salmon. All adults were intercepted at the weir, with hatchery (supplementation) adults passed upstream to spawn naturally at a level that numerically does not exceed the wild/ natural component, as part of the ISS experimental design. This project utilizes the here-to-for unrealized potential of genetically identifying every adult passed over the Pahsimeroi weir of both wild and hatchery origin and subsequently identifying their offspring as they pass through the system on their outward migration.

The ISS project also funds adult and juvenile monitoring activities for numerous other spring and summer chinook populations sites including the population that spawns naturally above the weir at Sawtooth Hatchery on the upper Salmon River. At the Sawtooth site, the ISS project enumerates and collects biological samples from all natural and supplementation adults that ascend the river and are allowed spawn above the weir. Project personnel conduct periodic foot surveys of spawning grounds above the weir to record numbers and distribution of spawners. They also sub-sample juveniles that migrate downstream past the weir site. Consequently, the infrastructure already exists to implement behavioral and genetic investigations on the Upper Salmon River identical to those already being conducted on the Pahsimeroi River.

Based upon the existing infrastructure at the ISS sites on the Pahsimeroi and Upper Salmon rivers and the demonstrated feasibility of the ongoing pilot project on the Pahsimeroi River, this proposal requests funding to expand Pahsimeroi investigations to the F_2 generation and to test models developed from this Pahsimeroi data to predict reproductive success of various crosses between fish that are allowed above the Sawtooth weir (Hatchery ♀X Hatchery ♂; Hatchery ♂X Wild ♀; Wild ♀ X Wild ♂; and Wild ♂ X Hatchery ♀). Thus, the much larger data set collected from interactions at Sawtooth would serve as a replication and validation of the work on the Pahsimeroi. The project described in this proposal, hereafter referred to as the Comparative Reproductive Success (CRS) project provides a unique opportunity since returns from future cohorts elsewhere are likely problematic with respect to sufficient numbers of adult fish to make

statistical comparisons. Moreover, since a pilot project already exists, methods detailing all behavioral and genetic analyses have already been worked out and the parental generations at both locations (Pahsimeroi and Sawtooth) have already been sampled. Thus, this study is capable of most cost-effectively addressing the questions specifically asked within the RFP:

- Are there statistically significant differences in reproductive success between natural-origin and hatchery-origin fish when measured at the first (F_1) and second generation (F_2)?
- Do F_1 progeny with HxW parents differ from F_1 progeny with HxH parents in the production of F_2 progeny?
- What are possible hypotheses to explain this difference? For example, can the difference be attributed to reduction in genetic fitness of hatchery-origin fish compared to natural-origin fish?
- Are differences more significant during any specific life history stages?
- What is the likely effect of any difference, in terms of population growth, population recovery, and genetic diversity/fitness in subsequent generations according to the Viable Salmonid Population (VSP) criteria?

PROJECT DESCRIPTION

Existing Infrastructure

Field activities supporting the CRS project will be integrated with the ISS project. As stated above, the ISS study presently maintains monitoring and evaluation activities in the Pahsimeroi River and the Upper Salmon River, as part of their study design but genetic evaluations proposed in the CRS project significantly expands on the scope of work of the ISS project. Equipment and personnel needed for performing a variety of ISS tasks (e.g. estimating juvenile chinook salmon outmigration and adult returns, collecting tissue samples) are located on site from mid-March through November. As the lead coordinating agency for ISS, the IDFG would function as the representative cooperator to CRS and provide logistical support for field sample and data collections.

ISS is an ongoing cooperative research project that was initiated in 1991 to evaluate supplementation as a recovery tool for Snake River chinook salmon stocks returning to Idaho. ISS research activities are distributed among four cooperative agencies that are financially supported by the Bonneville Power Administration (contract numbers; 1989-089-00, 1989-089-01, 1989-089-03, 1989-089-04). Presently, the research is entering the evaluation phase. Following completion of a programmatic review and statistical treatment of ISS data for review by the Independent Science Review Panel, new study timelines were developed (Lutch et al. 2003). Further, recommendations were made for evaluating an additional generation of chinook salmon and extending the project through 2012.

The significance of the ISS study to CRS relates directly to objectives in the ISS study design that focus on evaluating the effects of supplementation/augmentation on existing wild/natural chinook salmon populations. Pursuant to these objectives are specific tasks that are currently identified in the ISS Statement of Work for CY 2003. For the purpose of evaluating changes in natural production and productivity of chinook salmon, the IDFG representative for ISS operates rotary screw traps at both locations to estimate juvenile production, applies weir management and escapement criteria for adults returning to study reaches above the satellite hatcheries, and enumerates escapement (redd counts, adult returns to weirs).

In 2002, the ISS study extended their research activities to more directly evaluate the affect of hatchery reared supplementation broodstock on chinook salmon productivity. The Pahsimeroi River and the Upper Salmon River were selected as case studies since escapement weirs are nearly 100% effective at these locations. The existing ISS infrastructure was used to collect tissue samples from adult chinook salmon released upstream to spawn naturally. Predictive power using forecasted numbers for adult returns and juvenile outmigration was examined prior to sample collections. Data were also collected to examine temporal and spatial aspects of spawning activity between hatchery and wild natural chinook salmon. Presently, this additional ISS research has provided adult tissue samples from all potential parentage combinations of naturally spawning chinook salmon for 2002. These samples are stored at the IDFG Genetics Laboratory, Eagle Hatchery, ID, and await funding sources for processing and analysis. Logistical support for CRS project will be provided directly through ISS research activities.

Laboratory activities supporting the CRS project will be integrated with ongoing genetic studies at the University of Idaho's Center for Salmonid and Freshwater Species at Risk which utilizes several, high-throughput, multiplex genotype sets of microsatellite loci specifically developed for chinook salmon. The center currently employs these molecular markers to address numerous genetic questions on chinook populations as project sponsor or subcontractor to several BPA funded projects including the Johnson Creek supplementation project, the Salmon River chinook salmon captive rearing research project, and others. The laboratory has already used all the molecular and statistical procedures outlined in the methods section to successfully conduct parentage analysis on spring/summer chinook salmon from the Pahsimeroi River. Research proposed under the CRS project significantly expands on the scope of work outlined in these other activities.

Study Design

Weirs in position on both the Pahsimeroi and Upper Salmon Rivers allow for sampling and enumeration of all returning adults with essentially 100% efficiency. This includes the parental generation, first filial (F_1) and second filial (F_2) returning adults to be examined in this project. Screw traps operated in these systems will allow for the timely capture of juveniles of different life stages to be sampled for genetic analyses. Parentage analysis (parental exclusion analysis) will be used to assign offspring back to parental crosses. Assignment need not be 100%, only robust enough to assign proportions of different possible crosses to juveniles and subsequent generations. If hatchery adults exhibit the same spawning success as their wild counterparts, and randomly interbreed, then the observed proportions of offspring from each possible cross should not be significantly different from the proportions of wild and hatchery fish among male and female adults placed over the weir(s). If however, mating is not random or there is differential spawning success between hatchery and wild fish, then this will manifest itself in two ways. First, non-random mating would be evidenced by observed genotypic proportions being out of Hardy-Weinberg equilibrium with expected heterozygous and homozygous genotypic proportions. Secondly, differential spawning success would also be observed in significant departures from the probabilities of expected, random crosses (i.e. if 70% of males and females placed over the weir are hatchery origin we would expect a similar proportion of juveniles from those parents in the F_1 population).

These types of analyses will be tested via reject-support type hypothesis testing as outlined in the methods section. Sample sizes required for statistical significance and power have been calculated. Parentage assignment also allows for an even greater detailed analysis of hatchery vs. wild spawning success since the success of males and females from each origin can be assessed.

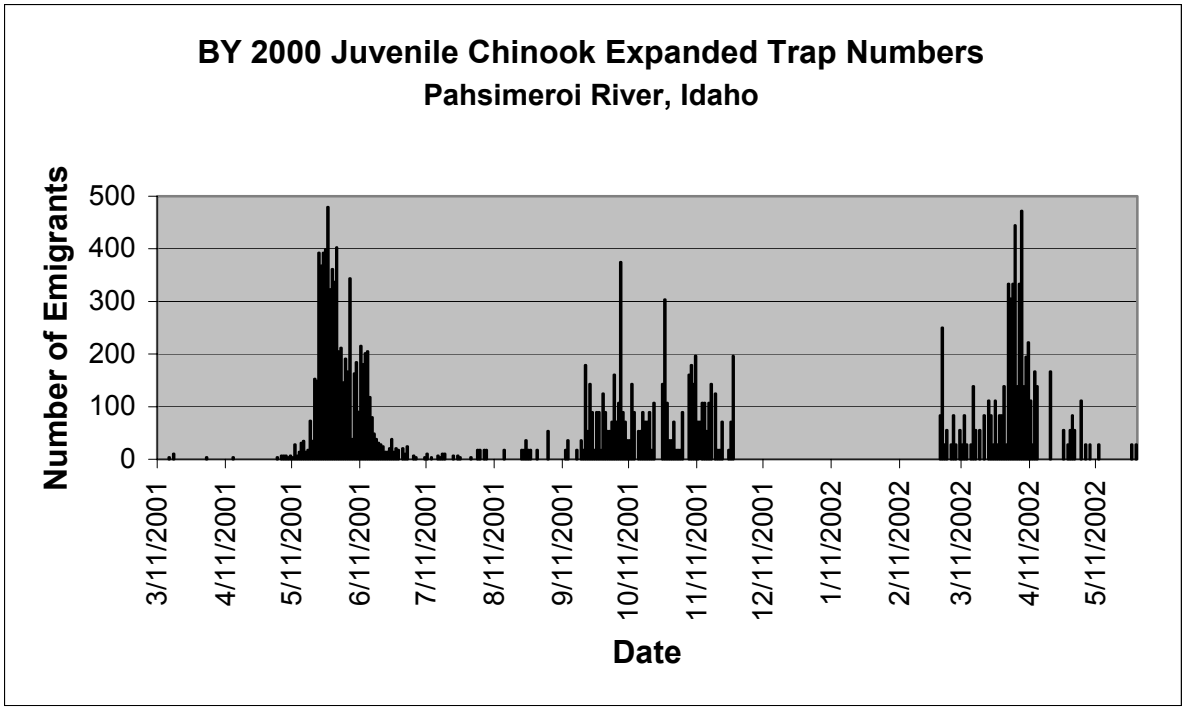

Figure 1. Expanded trap numbers and timing for juvenile chinook in the Pahsimeroi River, ID.

Thus, it may be for instance that wild males contribute more to the F_1 generation than hatchery males regardless of the female's origin. Alternatively, hatchery males may be preferentially selected by hatchery females thus providing evidence that hatchery fish selectively breed amongst themselves rather than with wild counterparts. All possible crosses and their departure(s) from random mating can be assessed.

Juvenile life stages will be sampled at more than one time since parr, presmolts and smolts are distinguishable on the basis of size and timing in these systems as in Figure 1. This design allows for the examination of changes through time of allelic and genotypic proportions in the juvenile population. For example, spawning success may not differ significantly between hatchery and wild origin parents and their crosses, but juvenile mortality or their timing may differ. Sampling the juvenile population at more than one life stage (parr = T_0, presmolt = T_1, smolt = T_2) allows for the detection of this potential differential success.

Critical Uncertainties and Study Rationale

Why use Pahsimeroi and Sawtooth stocks as opposed to ESU's listed in the RFP?
The best science would provide not only information about a biological system but also infer a set of predictive outcomes given similar circumstances. Thus, information gathered from one system could be extrapolated to another. Alternatively, if the information obtained is not predictive, then each system must be evaluated on a case-by-case basis. Whether interactions between hatchery and wild salmon and any resultant differential success can be predicted across different systems remains unknown. Studies examining hatchery influence on wild populations have been used to predict the interaction on other systems but none have been empirically tested. Ongoing, BPA funded projects addressing specific Snake River spring/summer chinook salmon ESU's under RPA 182 have not been replicated. Pahsimeroi and Sawtooth chinook salmon stocks were

selected for this proposal because of the following criteria: 1) adequate sample sizes through the F_2 generation for detection of specific crosses in returning adults, 2) small enough sample sizes and geographic area for the study to be 'manageable' in size, 3) populations with sufficient numbers in parental returns such that density dependent effects and/or Allee effects are minimized, 4) allogenic factors or outside influence from different alleles (i.e. straying) are minimal, 5) existing infrastructure of weirs and collection equipment, 6) a collaborative, ongoing, long-term evaluation of population dynamics and ecology in those systems (ISS), and 7) an ongoing pilot project (Pahsimeroi) from which information can be used to predict the outcome of a much larger, replicated data set (Sawtooth).

Will there be sufficient returns in coming years to insure adequate numbers of fish to examine statistically? Principal evidence for differential reproductive success between hatchery origin and wild natural chinook salmon lies in the ability to detect relative differences in fitness variables (e.g. survival) measured between the two groups (Roff, 1997) [also see (Endler 1986) for a comprehensive review of methods for detecting differential fitness in the wild]. The power to detect such differences depends largely on adequate sample sizes for detection of all combinations of parental crosses (e.g. Hatchery ♀X Hatchery ♂; Hatchery ♂X Wild ♀; Wild ♀ X Wild ♂; and Wild ♂ X Hatchery ♀). As a first step to predicting statistical power for this project, adult escapement was forecasted for wild natural and hatchery origin chinook salmon through 2012 at both Pahsimeroi and Sawtooth weirs. As demonstrated in Table 1, sufficient chinook salmon adults representing both groups are expected to return through the adult sample collection phase. Using these estimates and applying a recruit per spawner estimate, the representative brood year outmigration of juveniles is also predicted to be adequate for second-generation (F_2) genetic analyses.

Adjustments to forecasts of adult escapement will be coordinated with the ISS study as more data become available (e.g. PIT tag data). Refined estimates will be also applied to sub-sampling methods for collecting juvenile chinook salmon in the second generation.

Has parentage analysis been used in similar studies? Yes. Parentage analysis has been used successfully in several other fish studies (Bernatchez and Duchesne 2000; Eldridge et al. 2002; Estoup et al. 1998; Letcher and King 2001; Norris et al. 1999; O'Reilly et al 1998) including chinook salmon from the Snake River (Stephenson submitted). All laboratory and data analysis methods required for this project have been successfully utilized by the Center for Salmonid and Freshwater Species at Risk.

Is there sufficient genetic variation between hatchery and wild components in the proposed stocks for parental exclusion to be useful? Yes. Current evidence from other ongoing projects in the region (Stephenson submitted) suggest more than sufficient genetic variation to conduct parentage assignment tests even from these closely related groups. In this instance, the population components of the supplementation program would likely be too close for population assignment but not for parental assignment. Similar work on estimates of relative survival between two groups of fish (hatchery vs. natural origin) has been successful using microsatellites to separate those two closely related groups (Eldridge et al. 2002).

If differential reproductive success is observed, will this study tell us why? No. This study is designed to detect statistically significant differential reproductive success among four potential genetic crosses with a high degree of power. It is not designed to examine possible causes for that differential reproductive success. All the intrinsic and environmental parameters (both stochastic and deterministic) that may affect reproductive success are beyond the scope of this project.

Table 1. Forecasted returns of adult chinook salmon and estimated juvenile production in the Pahsimeroi River and the Upper Salmon River, return years 2003 - 2012. Adult forecasts based on brood year production estimates and smolt to adult return rates of 0.6 % for Pahsimeroi River and 0.5 % for Upper Salmon River. Brood Year Juvenile production estimates are calculated from expected wild/natural and hatchery females released above escapement weirs, then applying parr/pre-smolt per female and smolt per female estimates specific to each stream. nr = no supplementation returns expected since ISS releases ceased with broodyear 2002.

	Adults		Juvenile Production	
	Wild/Natural	Hatchery	Parr, Pre-smolts	Smolts
Pahsimeroi R.				
2003	154	361	49,126	19,866
2004	108	378	34,452	13,932
2005	374	609	119,306	48,246
2006	362	404	115,478	46,698
2007	323	100	67,469	27,283
2008	226	nr	36,047	14,577
2009	785	nr	125,208	50,633
2010	760	nr	121,220	49,020
2011	444	nr	70,018	28,638
2012	237	nr	37,802	15,286
Upper Salmon R.				
2003	128	171	64,512	20,736
2004	318	375	160,272	51,516
2005	914	434	339,696	109,188
2006	1,248	473	461,160	148,959
2007	205	118	81,396	26,163
2008	509	nr	128,268	41,229
2009	1,078	nr	271,656	87,318
2010	1,377	nr	347,004	111,537
2011	258	nr	65,016	20,898
2012	407	nr	102,564	32,967

Can results from this study be extrapolated to other systems or ESU's? Unknown. Presumably, if results obtained from the Pahsimeroi system can successfully be used to predict hatchery vs. wild interactions in the Sawtooth system, this would provide evidence that such interactions are indeed predictable. Whether this extends across chinook stocks out of the Snake River or to different species such as steelhead would require additional studies.

Objectives and Testable Hypotheses

Objective 1.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of F_1 smolts. *Since tissue samples will be collected from smolts through 2009 (Table 2), analysis of reproductive success to F1 smolt production will be replicated using brood year 2002 through 2005.*

Testable hypothesis: *There are no significant differences in the reproductive success of hatchery and natural origin parents to the production of F_1 smolts.*

Task 1.1 Collection of adults (parental types). *Importantly, all adults allowed above the weir in 2002 (hatchery and natural origin) have already been sampled and resulting F_1 progeny will be representatively sampled as smolts in 2004.*
All returning, pre-spawn adults collected and passed over the Pahsimeroi (n=299) and Sawtooth weirs (n=1340) in the fall of 2002 (differentially marked and unmarked males and females) were genetically sampled. All tissue samples have been stored in lysis buffer at the Eagle Fish Genetics Laboratory pending genetic analysis.

Task 1.2 Collection of smolts.
A sub sample of smolts originating from parents spawning above the Sawtooth weir in 2002 will be collected as they emigrate past the Sawtooth juvenile trap site in 2004 ($n \geq 460$). Smolts will also be collected at the Pahsimeroi juvenile trap site in 2004 ($n \geq 460$) from parents that spawned above the Pahsimeroi weir in 2002. All tissue samples will be stored in lysis buffer at the Eagle Fish Genetics Laboratory pending genetic analysis.

Task 1.3 Generation of genetic data and analysis.
Genomic DNA will be extracted from tissues samples taken from adults and juveniles. Multilocus genotypes of all adults and juveniles will be generated using highly polymorphic microsatellite loci. Juveniles will be assigned back to individual parents using maximum likelihood, and Bayesian procedures to exclude adult genotypes. The expected proportions versus observed proportions of parents contributing to the smolt population will be compared statistically. Funds are not being requested for the genetic analysis of Pahsimeroi adults sampled in 2002 or Pahsimeroi juveniles sampled in 2003 and 2004.

Objective 2.0 Determine parental proportions among resulting F_1 progeny at the parr, presmolt and smolt life stages. *Parental proportions will be analyzed using brood year 2002-2005 production data.*

Testable hypothesis: *Parental proportions are not significantly different among F_1 progeny life stages (parr, presmolt and smolt).*

Task 2.1 Collection of various juvenile life stages.
Genetic samples from parr (n>90), and presmolt (n>90) life stages from parental spawning in 2002 are currently being collected. As stated above, smolts from the same 2002 parental spawning will be collected in 2004.

Task 2.2 Generation of genetic data and analysis of various juvenile life stages.
Using the same procedures in Task 1.3 above, juveniles will be assigned to individual parents. The expected proportions vs. observed proportions of F_1 progeny at different life stages will be statistically compared.

Objective 3.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of F_1 adults. *Since tissue samples will be collected from adults through 2012 (Table 2), analysis of reproductive success to F1 adult production will be replicated using brood year 2002 through 2005.*

Testable hypotheses: *There are no significant differences in relative reproductive success of hatchery and natural origin parents to resultant F_1 adults.*

There are no significant differences in relative reproductive success of hatchery and natural origin F_1 adults from juvenile life stages of the same year class.

Task 3.1 Collection of returning F_1 adults.
Genetic samples of all F_1 adults originating from the 2002-2005 parental crosses will be collected from 2005 to 2010 as they return to the Pahsimeroi and Sawtooth weirs.

Task 3.2 Generation of genetic data and analysis of various F_1 year classes.
Using the same procedures in Task 1.3 above, F_1 adults will be assigned to individual parental crosses. The expected proportions vs. observed proportions of F_1 adults will be statistically compared to the parental crosses and to the proportion of genotypes present in different juvenile life stages.

Objective 4.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of juveniles and adults when measured at the second generation (F_2 juveniles and adults).

Testable hypothesis: *There is no significant difference in relative contribution of hatchery and natural origin parents to resultant F_2 smolts.*

Task 4.1 Collection of various juvenile life stages.
Genetic samples of emigrating parr (n>90), presmolt (n>90), and smolt ($n \geq 460$) life stages (F_2s) will be collected from the Pahsimeroi and Sawtooth systems from 2006 to 2009, in the same manner as they were collected in 2003 and 2004.

Task 4.2 Collection of F_2 adults.
Genetic samples of F_2 adults returning to the Pahsimeroi and Sawtooth weirs will be collected from 2008 to 2010.

Task 4.3 Generation of genetic data and analysis of various F_2 juveniles and adults.
F_2 juveniles and adults will be assigned to individual parents (F_1s) sampled as part of Objective 3.0. The expected proportions vs. observed proportions of F_2 progeny at different life stages will be statistically compared.

Sampling Methods

Adults-

Fin-clips were sampled from all returning adults allowed above the Pahsimeroi and Sawtooth weirs during the summer and fall of 2002 (Tables 3 and 4). Subsequently, non-lethal fin tissue will be sampled from all adult chinook salmon that return to the Pahsimeroi and Sawtooth weirs

Table 2. Summary of F1 and F2 life history stage present in each sampling year for cohorts originating from brood year 2002, 2003, 2004, and 2005.

1/ Cell labeling conventions are: F = filial, * = generation (I.e. F1 or F2), and, ** denotes age of fish (0+ and 1+ are parr/presmolt and smolt respectively, 3,4, & 5 are adults)

2/ Stippled juvenile cells occur in occur among F2's when the juveniles present include production that results from F1 jack that cross with adults from a preceding brood year that is not included in this study. For example the stippled juvenile cell for sample year 2005 indicates that age 0 juveniles are present that result from brood year F1 male jacks crossing with adults from brood years 2001 or 2000. These are not pure F2 fish from brood year 2002 but should be sampled.

3/ Stippled adult cells indicate sample years when adult returns are present that are unrelated to the brood year at the top of the column but must be sampled to track parentage of brood year 02 fish through the F2 generation or to track F1 adult returns from brood years 03, 04, and 05.

4/ ***Note that analysis through F2 for brood year 2002 requires sampling and analysis of juvenile for 03-09 and adults from 02-12. These same collected and analyzed samples can be used to complete F1 juvenile and adult analyses for brood years 30, 04, and 05 as well at no additional cost. Gray shaded sample years are not included in this proposal but if sampling were completed in these years, analyses of F2's from brood years 03, 04, and 05 could be also completed.***

during the summer and fall of 2003-2012 (Table 2), (samples in 2003 and 2004 are collected exclusively in relation to the ISS study, whereas samples from 2005-2012 will be collected since they will contain a proportion of fish that originate from 2002 parental spawners and also relevant to the CRS project).

Table 3. Sex and origin of chinook released above Pahsimeroi Weir.

Origin	Males	Females
Natural	**91**	**66**
Supplementation	**46**	**96**
TOTAL	**137**	**162**

Table 3. Sex and origin of chinook released above Sawtooth Weir.

Origin	Males	Females
Natural	480	314
Supplementation	236	310
TOTAL	**716**	**624**

Juveniles (parr, pre-smolts, and smolts)-

Rotary screw traps were installed near the Sawtooth Hatchery and Pahsimeroi Hatchery weirs in March 2003 to sample emigrating juvenile chinook. Fin samples from brood year 2002 production will be collected during 2003 and 2004 from three discernable life stages as they migrate downstream past these traps: parr will be sampled from .May through July, presmolts will be sampled from September through November, and smolts will be sampledthe following spring in March through mid April. Because there may be inherent differences in parental contribution to the three groups, they will be treated separately. In order to obtain a representative sample from all production above the weir, sampling will be conducted proportionally across the entire out migration (spring 2003 thru spring 2004). The exception will be the three-month period (December-February) when the trap will not be operational. During this period, very little movement of juveniles occurs. In order to accurately sample juveniles proportionately across the migration period, historical records of emigration timing (10 years) collected from ongoing production research by Idaho Fish and Game will be employed. Similar collections of parr, pre-smolts, and smolts will take place from 2005 to 2009 (Table 2).

Sample Sizes

To compare the relative distribution of crosses contained in the sample set, the distribution of alleles, and the contribution of individual parents for the specific number of adults passed over the Sawtooth and Pahsimeroi weirs requires extensive sampling of smolts. A sample size of n=460 was chosen for smolt collection using the Power analysis program in STATISTICA (Statsoft Inc.) and information on the proportions of each group released above the Pahsimeroi and Sawtooth weirs in 2002, as well as anecdotal behavioral evidence. A putative hatchery ♂X wild ♀ cross is expected to have the smallest probability of occurrence and therefore detection. Thus, sample sizes were based upon the low probability of this cross occurring. Power analysis indicated that a sample size of $n \geq 460$ would allow the observation of all possible alternate outcomes of crosses occurring with a frequency as low as 1% with 99% accuracy. It would also

allow the detection of changes from expected frequencies at a true difference of >1% with 95% accuracy while maintaining 88% power.
In testing whether parental proportions are significantly different among F_1 progeny life stages (parr, presmolt and smolt), sampling effort will not need to be as intensive since we wish only to compare the relative proportions of crosses as they change or remain unchanged through time. In this instance a sample set of at least 90 individuals will ensure we can detect changes in the proportions of crosses with greater than 95% probability.

Genetic Analyses

DNA will be extracted following a Qiagen tissue protocol (Qiagen Laboratories). Ten to twelve microsatellite loci will be amplified for each individual following procedures outlined by (Narum et al. *submitted*; Williamson et al. 2002). These loci have demonstrated high levels of allelic variation and heterozygosity in chinook salmon populations (Table 2, adapted from Williamson et al. 2002).

Locus	**Repeat motif of original clone**	**Allele size range (bp)**	**No. of alleles**	**Heterozygosity**		
				H_O	H_E	**HWE**
OtsG3	$(GAAT)_8$-GATAGATTAATA-$GATA)_{11}$-GATTAATAGAGA-$(GATA)_{26}$	146-246	5	0.33	0.37	ns
OtsG68	$(GATA)_{30}(TAGA)_1$	184-296	12 (17)	0.88	0.97	ns
OtsG78b	$TAGA(TATA)_2$-N_{12}-$(TAGA)_{31}$	216-356	13	0.88	0.95	ns
OtsG83b	$(TGTC)_7$-N_{51}-$(TATC)_{34}$	155-303	15	1.0	0.98	ns
OtsG243	$(TAGA)_{63}(CAGA)_{12}(GACA)_7(GA)_{22}$	190-466	12	1.0	0.96	ns
OtsG249	$(TAGA)_{19}$	192-310	13 (14)	1.0	0.95	ns
OtsG253b	$(GACA)_{10}$ $(GATA)_{14}$	141-301	12	1.0	0.96	ns
Ots311	$(GATA)_{30}$-GACA-$(GATA)_2$-$(GAGTGATA)_7$-GATA	278-374	12	0.88	0.95	ns
OtsG409	$(GA)_9(TAGA)_6$-GGTA-$(GATA)_{16}$	116-282	10	0.77	0.91	ns
OtsG422	$(GATA)_{24}$	264-414	15	1.0	0.97	ns
OtsG432	$(GATA)_3$-GGAT-$(GATA)_8$	122-202	12	0.88	0.95	ns
OtsG474	$(GATA)_6$	155-191	6	0.66	0.75	ns

Products from PCR amplification will be run out on an ABI 3100 Genetic Analyzer (Applied Biosystems). Allele sizes and genotypes will be determined using the software programs Genescan 3.0 and Genotyper 2.1 (Applied Biosystems).

Statistical Methods

Juveniles will be assigned to parental crosses via comparison of multilocus microsatellite genotypes among candidate parents. Maximum likelihood (Marshall et al. 1998) and Bayesian (Neff et al. 2001; Lange 1997) procedures will be used to exclude possible crosses and parents (parental exclusion analysis). Observed versus expected parental contributions will be

analyzed with Goodness-of-fit tests ($\chi 2$, Fisher's Exact Test, G-Test) (Motulsky 1995; Zar 1996). Differences among life stages will be analyzed with paired t-tests between groups (parr, presmolt, smolt) (Motulsky 1995; Zar 1996). Changes in allele and/or genotypic frequencies will be examined using statistical software for population genetics [Genepop (Raymond and Rousset 1995); GDA (Lewis and Zaykin 1999)] and a Bayes estimation of allele frequencies (Dirichlet-multinomial distributions) to assess linkage and provide predictive distributions (Lange 1997).

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Timeline

September 2002	Fin-clips samples collected from all adults passed above Pahsimeroi (N=299) and Sawtooth (N=1340) weirs **(COMPLETED)**
Mar 2003-Dec 2004	Sample F_1 parr (n>90 at each site), F_1 pre-smolts (n>90 at each site) (ONGOING) and F_1 smolts (n≥460) at Pahsimeroi and Sawtooth for genetic fin-clips
January 2004	Completion of data collection of 10-12 microsatellite loci on 2002 adults from Pahsimeroi (N=299) and adults from Sawtooth (N=1340), **preliminary report to BPA on project status**
August 2004	Completion of data collection of 10-12 microsatellite loci for F_1 parr, F_1 pre-smolts (n≥90) and F_1 smolts (n≥460) from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_1 parr, pre-smolts, and smolts**
Jun 2005-Oct 2010	Fin-clips samples collected from all adults passed above Pahsimeroi and Sawtooth weirs can be used to assess reproductive success to F_1 for brood year 2002 as well as 2003-2005.
Mar 2006-Dec 2009	Sample F_2 parr (n>90 at each site), F_2 pre-smolts (n>90 at each site) and F_2 smolts (n≥460) at Pahsimeroi and Sawtooth for genetic fin-clips
January 2008	Completion of data collection of 10-12 microsatellite loci on F_1 adults from Pahsimeroi (N=299) and adults from Sawtooth (N=1340), **preliminary report to BPA on project status**
August 2008	Completion of data collection of 10-12 microsatellite loci for F_2 parr(n≥90) and F_2 pre-smolts (n≥90) from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 parr and F_2 pre-smolts**
January 2010	Completion of data collection of 10-12 microsatellite loci for F_2 smolts from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 smolts**
Jun 2008-Oct 2012	Fin-clips samples collected from adults for F_2 analysis of production in Pahsimeroi and Sawtooth rivers originating from 2002 parental crosses.
December 2012	Completion of data collection of 10-12 microsatellite loci for F_2 adults from Pahsimeroi and Sawtooth, **completion report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 adults**

Facilities and Equipment

Only limited field equipment costs and tissue collection costs are necessary during the entire term of this project. Adult tissue samples were already been collected in 2002 and current juvenile collections are supported by the existing ISS budget.

The genetic work described in this proposal will be conducted out of the Idaho Department of Fish and Game's fish genetics laboratory at Eagle, Idaho and the Salmonid and Freshwater Fish Genetics Research Laboratory at the University of Idaho's Hagerman Fish Culture Experiment Station. Between the two facilities all of the necessary molecular genetic analysis equipment and expertise for this work is already in place.

The only capital equipment requested for this project is a centrifuge to run 96 well PCR plates and a PCR thermal cycler. We are also requesting as part of operating expenses, the lease of a ABI 3100 fragment analyzer to expedite the generation of multilocus, microsatellite genotypic data for approximately 3000 genetic samples. The ABI 3100 fragment analyzer currently owned and in operation at the University of Idaho Hagerman's laboratory can complete all proposed analyses. However, the timeliness of the project would be greatly facilitated by the lease of an additional instrument for a fixed period of time. Analyses of costs associated with personnel and equipment indicates the lease of an additional instrument would be more cost effective than the retention of extra personnel throughout the year to operate a single instrument.

Qualifications of Participants

Dr. Madison Powell received his Ph.D. in the Systematics & Evolutionary Biology program at Texas Tech University in 1995 and is currently an Assistant Professor in the Department of Fish and Wildlife Resources and Department of Animal and Veterinary Sciences at the University of Idaho. Dr Powell is also the director of the Center for Salmonid & Freshwater Species at Risk at the University of Idaho. He supervises UI molecular genetic laboratories at the Aquaculture Research Institute in Moscow, ID and at the Hagerman Fish Culture Experiment Station in Hagerman, Idaho. The laboratories' primary goals are to provide timely genetic information to applied conservation genetic questions, and provide genetic advice and consultation to state, federal, and tribal agencies regarding endangered fishes and fisheries management. Dr. Powell is currently the Principal investigator of several genetic projects examining reproductive success of hatchery and wild fish using microsatellite DNA analyses including (sockeye project BPA #199107200), and chinook captive broodstock project BPA #199009300). Dr. Powell will assist in the development of the research study design, supervise genetic lab work, analyze data and report results.

Education
Ph.D. Zoology, Texas Tech University (1995)
M.S. Zoology, University of Idaho (1990)
B.S. Zoology/Biology, University of Idaho (1985)
Expertise:
Fishery/Genetics Research:
UI Assistant Professor researching conservation genetics of salmonids (2 years)
Expertise Specific to this Project:
UI Research Scientist studying endangered sockeye populations in Snake River ID (7 years)
Dissertation using genetic fragment analysis to discriminate populations

Matthew Campbell (IDFG) is currently employed by IDFG as a fisheries biologist/geneticist, and oversees genetic projects at IDFG's Eagle Fish Genetics Lab. Current projects include using microsatellite analyses to assess the reproductive success of hatchery and wild chinook salmon at the Pahsimeroi River and to assess the reproductive success of hatchery and wild spawning sockeye salmon at Redfish Lake, ID. Matt received a M.S. degree in Fisheries (emphasis in genetics) from the University of Idaho, examining hybridization and introgression issues in cutthroat trout populations using molecular markers. He previously worked at the University of Idaho's genetics lab for over six years examining hybridization, genetic diversity, and genetic population structure of fish species throughout the Pacific Northwest using mtDNA and microsatellite DNA analyses. Matthew Campbell will perform genetic work with assistance from 1 scientific aide and will assist Matt Powell with data analysis and reporting of results.

Education:
BSc (Fisheries Research) from University of Idaho (1995)
MSc (Fisheries Research-emphasis in fish genetics) from University of Idaho (2001)
Expertise:
Population Genetics Research:
IDFG geneticist-current
University of Idaho – Biological Aide (Genetics Lab), Center for Salmonid and Freshwater Species at Risk (5 years)-Moscow, ID
Expertise Specific to this Project:
Supervises State's chinook salmon genetic projects
Proficient in generating and analyzing microsatellite data on a ABI 310 and ABI 3100 fragment analyzer.

Jeffrey Lutch is a Senior Fishery Research Biologist with the IDFG at the Nampa Research Facility. As the lead biologist for the Idaho Supplementation Studies project, he is evaluating benefits and risks of different chinook salmon supplementation strategies on natural production and productivity. Jeff was previously employed as a fishery biologist with the National Park Service in Yellowstone National Park, where he performed status assessments for cutthroat trout populations while documenting the extent of genetic hybridization with non-native salmonids. Previously, he worked as a fishery biologist with Bureau of Land Management in Alaska, and studied the affects of recreational use on fisheries. Jeff received his B. S from the University of Pittsburgh, and an M. S. from Clarion University, where he investigated aggressive interactions between native and introduced trout and the effects on reproductive success. Jeffrey Lutch will coordinate sample and data collections supported by the Idaho Supplementation Studies project, and will assist in data analysis and report writing.

Education
BS. (Biology) from the University of Pittsburgh (1990)
MS in Biology (emphasis in fish ecology) from Clarion University of Pennsylvania (1994).
Fishery Research Expertise
Species Interactions
Population Dynamics
Hatchery Supplementation
Exotic species control
Recreational Fisheries
Population monitoring and evaluation

Expertise to this project
Thesis examining reproductive success of sympatric native and introduced salmonids
Coordinates the Idaho Supplementation Studies project
Proposed and supervises the small-scale reproductive success study between hatchery and wild chinook salmon at the Pahsimeroi River.

Sam Sharr is currently Principal Research Biologist for anadromous fish at IDFG and supervises the implementation of the *Idaho Natural Production Project* (NPP) for chinook and steelhead, the *Idaho Salmon Supplementation* (ISS*)* Project for spring and summer chinook salmon, and the *Idaho Steelhead Supplementation* (SSS) Project. These Bonneville Power Administration funded projects monitor natural populations of chinook and steelhead and evaluate the efficacy of supplementation as a restoration tool for salmon and steelhead populations. Sam has a B.S. degree in Biology from the University of Washington and completed additional studies while working at the University of Wisconsin Limnology Laboratory. He subsequently spent 16 years with the Alaska Department of Fish and Game conducting population monitoring and life history studies on salmon and herring populations and damage assessment research on salmon populations impacted by the *Exxon Valdez* oil spill. He has also worked as a salmon research biologist for the Hoopa Valley Tribal Fisheries Department in California and as the Ocean Salmon Fisheries Manager for the Oregon Department of Fish and Wildlife. In the latter role, he had a lead role in developing a large monitoring program for coastal fall chinook populations.

Education:
BS (Biology) from University of Washington (1972)
Expertise:
Fisheries Research:
IDFG Principal Research Biologist – Anadromous Fish (current)
Hoopa Valley Tribe - Fisheries Research Biologist (2 years)
Alaska Department of Fish and Game (16 years)
- Principal Investigator - salmon damage assessment research conducted in response to the Exxon Valdez Oil Spill.
- Prince William Sound Area Research Biologist - salmon and herring.
- Statewide Stock Biology Research Biologist – salmon and herring.

ODFW (4 years)
- Supervised development of an integrated escapement indicator and harvest rate indicator stock monitoring programs for Oregon Coastal fall chinook.

Fisheries Management
ODFW Ocean Salmon Fisheries Manager
- Ocean harvest modeling
- Technical Committees of the PSC and PFMC

Expertise specific to project:
Stock identification research for salmon and herring.
- Scale patterns analysis
- Coded wire tagging studies

Allozyme based GSI and stock structure research

Budget

Reproductive Success of wild and hatchery chinook salmon-BPA FY2003 (IDFG 2004)

Personnel Costs						
	Comments	Salary/hr	Hours/week	Weeks		Total
Temporary	Genetic lab assistant	$18.00	40	32	$	23,040
	Assistant benefits (35.0%)				$	8,064
Temporary	(2) Techs. for trap op., sample collection	$11.88	40	32	$	30,413
	Tech benefits (42.8%)				$	13,017
Temporary	(2) Bioaides for trap op., sample collection	$7.63	40	32	$	19,533
	Tech benefits (49.8%)				$	9,727
				Total Personnel Costs	$	103,794
Operating Costs						
Supplies (not Cap Outlay)	Chemicals, pipet tips, gloves, usat primers, etc	Cost/sample	# of samples			
HFCES (UofI)	DNA extractions, quantifications, normalization	$2.00	1900		$	3,800
Eagle Genetics Lab (IDFG)	PCR amplifications, usat electrophoresis	$19.00	1900		$	36,100
Equipment Lease	ABI 3100 fragment analyzer (2003-2007)				$	41,058
Misc.	Equipment repair, misc.				$	5,000
				Total Operating Costs	$	85,958
Capital Outlay Costs						
1 PCR machine (2003)					$	5,000
1 Centrifuge (2003)					$	8,000
				Total Cap Outlay Costs	$	13,000
				Subtotal	$	202,752
				Overhead (20.9% of operating and personnel)		39,658
				Total Costs	**$**	**242,410**

Estimated costs through 2012

Cost estimates (2004-2012)	TOTAL
2004	**$233,137.00**
2005	**$259,261.00**
2006	**$269,068.00**
2007	**$247,144.00**
2008	**$206,086.00**
2009	**$206,086.00**
2010	**$179,206.00**
2011	**$179,206.00**
2012	**$179,206.00**